Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Parasitology 133 (2005) 299–306
veterinary parasitology
www.elsevier.com/locate/vetpar

Genetic and biologic characteristics of *Toxoplasma gondii* infections in free-range chickens from Austria

J.P. Dubey [a,*], R. Edelhofer [b], P. Marcet [c], M.C.B. Vianna [a], O.C.H. Kwok [a], T. Lehmann [c]

[a] *United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA*
[b] *Institut fur Parasitologie und Zoologie, Veterinärmedizinische Universität Wien, Veterinärplatz 1, A-1210 Wien, Vienna, Austria*
[c] *Division of Parasitic Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, MS: F22, Chamblee, GA 30341, USA*

Received 10 March 2005; accepted 7 June 2005

Abstract

The prevalence of *Toxoplasma gondii* in free-ranging chickens is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. The prevalence of *T. gondii* in free-range chickens (*Gallus domesticus*) from 11 Biofarms in Austria was determined. Antibodies to *T. gondii* assayed by the modified agglutination test (MAT) were found in 302 of 830 (36.3%) chickens with titers of 1:10 in 50, 1:20 in 69, 1:40 in 53, 1:80 in 40, 1:160 or higher in 90. Hearts of 218 chickens with MAT titers of 10 or higher were bioassayed individually in mice. Tissues from 1183 chickens were pooled and fed to 15, *T. gondii*-free cats. Feces of the cats were examined for oocysts; 11 cats shed *T. gondii* oocysts. *T. gondii* was isolated from 56 chickens by bioassay in mice. Thus, there were 67 isolates of *T. gondii* from these chickens. Genotyping of these 67 isolates using the SAG2 locus indicated that all 33 were Type II. Phenotypically and genetically these isolates were different from *T. gondii* isolates from Brazil. None of the isolates was virulent for mice. This is the first report of isolation of *T. gondii* from chickens from Austria.
Published by Elsevier B.V.

Keywords: *Toxoplasma gondii*; Chickens; *Gallus domesticus*; Free-range; Austria; Genotype

1. Introduction

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts,

* Corresponding author. Tel.: +1 301 504 8128; fax: +1 301 504 9222.
E-mail address: jdubey@anri.barc.usda.gov (J.P. Dubey).

0304-4017/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetpar.2005.06.006

or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is unknown whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or to other factors.

T. gondii isolates have been classified into three genetic types (I, II, III) based on restriction fragment length polymorphism (RFLP) (Howe and Sibley, 1995; Howe et al., 1997; Mondragon et al., 1998; Owen and Trees, 1999; Fuentes et al., 2001; Grigg et al., 2001; Ajzenberg et al., 2002; Boothroyd and Grigg, 2002; Jungersen et al., 2002; Aspinall et al., 2003; Ajzenberg et al., 2004; Dubey et al., 2004a,d; da Silva et al., 2005). The parasite used to be considered clonal with very low genetic variability. However, most of the information was derived from isolates from Europe and North America. Based on newer markers for genetic characterization and using recently isolated strains from Brazil and French Guiana revealed higher genetic variability than previously reported (Ajzenberg et al., 2004; Lehmann et al., 2004).

We have initiated a worldwide study of *T. gondii* population structure. For this we have chosen the free-range chicken as the indicator host for soil contamination with *T. gondii* oocysts because they feed from the ground. Thus far, we have characterized strains from South America [Brazil (Dubey et al., 2002, 2003a,d), Peru (Dubey et al., 2004b), Venezuela (Dubey et al., in press-f), Argentina (Dubey et al., 2003e; Dubey et al., in press-c)], Central America and the Caribbean [Guatemala (Dubey et al., in press-b), Grenada, West Indies (Dubey et al., in press-e)], North America [USA (Dubey et al., 2003c; Lehmann et al., 2003), Mexico (Dubey et al., 2004c)], Africa and Middle East [Egypt (Dubey et al., 2003b), Israel (Dubey et al., 2004e), Mali, Kenya, Burkina Faso, and Democratic Republic of Congo (Dubey et al., 2005a)], and Asia [Sri Lanka (Dubey et al., in press-d), India (Sreekumar et al., 2003)]. These studies are still not complete, nevertheless, a pattern is emerging that isolates from South America are genetically distinct (Lehmann et al., 2004).

Little is known of the characteristics of isolates of *T. gondii* from animals from Austria. In the present paper, we attempted to isolate and genotype *T. gondii* from chickens from Austria.

2. Materials and methods

2.1. Naturally-infected chickens

Samples were obtained from slaughter houses that catered to chickens from Bio-farms that were at least 2 km apart (Table 1). Samples were collected from the assembly line that processed 4000 chickens per hour. Therefore, it was not possible to match blood and heart from many chickens. From many chickens only hearts were collected. All chickens sampled were more than 1-year-old. Samples were obtained in three batches in July and August 2004, and January 2005. In the first batch (farms A–D) there were 276 chickens from four farms (Table 1). Blood and hearts were collected from 190 chickens; from the remaining 86 chickens from farms A and B blood and fluids were squeezed out of the hearts. In the second batch samples were collected from 936 chickens from five farms (farms E–I); from 396 chickens there were matching hearts and blood samples and only hearts were collected from 540 chickens (Table 1). In the third batch sera and matching hearts from 158 chickens and 225 hearts without sera were collected (Table 1). From two farms (J,K) sera and hearts were sent cold by air to the Animal Parasitic Diseases Laboratory (APDL), USDA, Beltsville, MD. Three or 4 days elapsed between killing of chickens and receipt of samples at Beltsville.

Chickens on these organic farms were maintained free-range; they all used centrally distributed feed (Bio food, Garant Co., Klagenfurt, Austria) and same type of management (outdoor access of 4 m^2 each animal with plants, stocking rates must not exceed 6 layers/m^2, 18 cm perches for each animal, and a maximum of 4800 chickens or 3000 layers in each poultry house). Chickens were kept indoors until 20-week-old and then roamed free on the pasture. The pastures had only wire fences and were not cat proof. Cats were present on farms, especially on farm H and cats from the neighbor hood had access to chicken pastures. All egg layers from a given farm were sent to slaughter and the premises were cleaned before starting a new batch. Chicken carcasses were not sold directly to consumers and were used mainly for baby food and soups.

Table 1
Seroprevalence of *Toxoplasma gondii* antibodies in chickens from 12 farms in Austria

Batch number	Farm code (Town)[a]	Coordinates long/lat		Number of chickens on the farm	Number of sera tested	Number positive	Titers						
							10	20	40	80	160	320	640
1	A (Stambach)	15°57′	47°20′	1411	47	10	2	2	2	2	2	ND	ND
	B (Kaindorf)	15°54′	47°13′	540	35	0	0	0	0	0	0	ND	ND
	C (St. Lorenzen)	15°57′	47°26′	1141	52	2	0	0	1	0	1	ND	ND
	D (Seitenstetten)	14°39′	48°02′	448	56	0	0	0	0	0	0	ND	ND
	A + B				86	5	1	0	1	0	3	ND	ND
2	E (Wolfsberg)	15°39′	46°50′	434	37	0	0	0	0	0	0	0	0
	F (Unterbergla)	15°18′	46°48	874	58	1	1	0	0	0	0	0	0
	G (Groß St. Florian)	15°19′	46°49′	392	47	40	20	11	6	1	1	1	0
	H (Wies)	15°16′	46°43′	732	131	125	9	15	30	26	16	13	16
	I (Weinitzen)	15°29′	47°08′	3142	123	5	5	0	0	0	0	0	0
3	J (Bad Gleichenberg)	14°39	48°02	430	57	22	5	6	2	2	1	6	ND
	J + K	15°54	46°52	1000	50	42	2	21	2	2	3	12	ND
	K (Riegersburg)	15°56	47°00	500	51	50	5	14	9	7	3	12	ND

[a] From Province Styria, except farm D from Lower Austria.

2.2. Serological examination

Sera of chickens were tested for *T. gondii* antibodies using serum dilutions; 1:10, 1:20, and 1:200 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). After the completion of the bioassays, all positive chicken sera were rerun using two-fold dilutions from 1:10 to 1:160 or higher (Table 1).

2.3. Bioassay of chickens for T. gondii infection

Tissues of most chickens were bioassayed for *T. gondii* infection 1–3 days after receipt in APDL.

Table 2
Isolation of *Toxoplasma gondii* from chickens by bioassay in cats

Batch number	Farm code	Number of chicken hearts fed	MAT titer of chickens	Cat number	Oocysts shed	*T. gondii* genotype	Isolate designation
1	A, B, C 1–3	122	<1:10	83	Yes	II	TgCkAt 1
	D, A, B 4, 0	137	<1:10	84	No		
2	H	75	Unknown	13	Yes	II	TgCkAt 2
	H	75	Unknown	34	Yes	II	TgCkAt 3
	G	45	Unknown	26	Yes	II	TgCkAt 4
	G	45	Unknown	88	Yes	II	TgCkAt 5
	F	75	Unknown	80	Yes	II	TgCkAt 6
	F	75	Unknown	46	Yes	II	TgCkAt 7
	I	75	Unknown	47	No		
	I	75	Unknown	22	No		
	E	37	<1:10	45	No		
3	J	57	Unknown	83	Yes	II	TgCKAt 8
		56	Unknown	79	Yes	II	TgCKAt 9
		56	Unknown	73	Yes	II	TgCKAt 10
		56	Unknown	89	Yes	II	TgCKAt 11

Table 3
Isolation of *Toxoplasma gondii* from chickens from farms A, B, and G in Austria

Farm code (chicken number)	Titer	Bioassay in mice	*T. gondii* genotype	Isolate designation
		Number of mice positive		
A, B (0-9)	≥160	4*	II	TgCkAt 12
A, B (0-13)	40	4	II	TgCkAt 13
A–1 (25)	≥160	5	II	TgCkAt 14
A, B (0-34)	≥160	5	II	TgCkAt 15
A, B (0-49)	≥160	5	II	TgCkAt 16
G (3-3)	40	3	II	TgCkAt 17
G (3-21)	80	5	II	TgCkAt 18
G (3-30)	40	5	II	TgCkAt 19
G (3-33)	160	5	II	TgCkAt 20
G (3-37)	320	5	II	TgCkAt 21

Number of mice positive out of five mice inoculated with chicken tissues.

Table 4
Isolation of *Toxoplasma gondii* from chickens from farm H by bioassay in mice

Chicken number	Titer	Bioassay in mice			*T. gondii* genotype	Isolate designation
		Primary passage	Subpassage			
		Tissue cysts seen	SW mice	KO mice		
4-4	160	1/1[a]	2/2	ND[b]	II	TgCkAt 22
4-7	40	0/1	2/2	1/1	II	TgCkAt 23
4-9	40	0/2	2/2	1/1	II	TgCkAt 24
4-13	160	1/2	ND[b]	ND	II	TgCkAt 25
4-20	160	1/2	ND	ND	II	TgCkAt 26
4-21	160	0/1	2/2	1/1	II	TgCkAt 27
4-24	≥ 640	1/2	ND	ND	II	TgCkAt 28
4-26	320	0/2	2/2	1/1	II	TgCkAt 29
4-31	≥ 640	0/2	2/2	1/1	II	TgCkAt 30
4-32	20	1/1	2/2	ND	II	TgCkAt 31
4-35	80	0/2	2/2	1/1	II	TgCkAt 32
4-47	80	0/2	2/2	1/1	II	TgCkAt 33
4-48	320	1/2	ND	ND	II	TgCkAt 34
4-52	≥ 640	0/2	2/2	1/1	II	TgCkAt 35
4-60	≥ 640	1/2	ND	ND	II	TgCkAt 36
4-75	80	0/1	2/2	1/1	II	TgCkAt 37
4-78	160	1/1	2/2	1/1	II	TgCkAt 38
4-80	320	0/1	2/2	1/1	II	TgCkAt 39
4-85	160	1/1	ND	ND	II	TgCkAt 40
4-90	320	1/2	ND	ND	II	TgCkAt 41
4-92	40	0/1	2/2	1/1	II	TgCkAt 42
4-93	40	0/2	2/2	1/1	II	TgCkAt 43
4-99	320	1/1	ND	ND	II	TgCkAt 44
4-104	320	1/1	2/2	ND	II	TgCkAt 45
4-117	160	0/1	2/2	1/1	II	TgCkAt 46
4-125	40	1/2	ND	ND	II	TgCkAt 47
4-126	320	0/1	2/2	1/1	II	TgCkAt 48

[a] Number of mice *T. gondii* positive out of two mice inoculated with chicken tissues. The mice that died within 7 days p.i. are not shown.
[b] ND: not done.

Hearts of 209 chickens with MAT titers of 1:10 or higher (16 in batch 1 with MAT of 1:10 or higher, 101 in batch 2 with titers of 1:40 or higher, 92 in batch 3 with titers of 1:20 or higher) were each bioassayed individually in out-bred female Swiss Webster (SW) mice obtained from Taconic Farms, Germantown, New York, as described (Dubey et al., 2002). Each heart was homogenized individually, digested in acidic pepsin, washed, and most of the homogenate was inoculated subcutaneously (s.c.) into two to five mice (Tables 2–5).

Tissue imprints of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 39 p.i. and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 41days p.i. and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). Brains of seropositive mice without demonstrable tissue cysts were inoculated s.c. into new SW mice and in 15 cases also in interferon gamma gene knock out (KO) mice (Table 3). The KO mice are highly susceptible to *T. gondii* infection (Dubey and Lindsay, 1998). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

Hearts from certain chickens were pooled (Table 2) and fed to 15 *T. gondii*-free cats (Dubey et al., 2002). Feces of the cats were examined for shedding of *T. gondii* oocysts 3–14 days post-ingesting chicken tissues as previously described (Dubey, 1995). Fecal floats were incubated for 1 week at room temperature to allow sporulation of oocysts and were bioassayed in mice (Dubey and Beattie, 1988). Mice were killed 5–8 days post-inoculation (p.i.) with oocysts or fecal floats and their mesenteric lymph nodes were examined microscopically for *T. gondii* tachyzoites and their homogenates were inoculated s.c. into new SW mice.

2.4. Genetic characterization for *T. gondii*

T. gondii DNA was extracted from mouse tissues or oocysts as described previously (Lehmann et al., 2000). The RFLP strain type of *T. gondii* isolates was determined by nested PCR on the SAG2 locus according to Howe et al. (1997).

Table 5
Isolation of *T. gondii* from chickens from Austria by bioassay in mice

Farm code	Chicken number	Titer	Number of *T. gondii* positive mice[a]	*T. gondii* genotype	Isolate designation
J	4	40	2	II	TgCkAt49
	8	≥320	4	II	TgCkAt50
	18	≥320	4	II	TgCkAt51
J + K	11	≥320	2	II	TgCkAt52
	15	160	4	II	TgCkAt53
	19	≥320	4	II	TgCkAt54
	28	≥320	3	II	TgCkAt55
	31	20	3	II	TgCkAt56
	42	≥320	3	II	TgCkAt57
	44	80	4	II	TgCkAt58
	48	40	4	II	TgCkAt59
	49	20	4	II	TgCkAt60
	50	≥320	1	II	TgCkAt61
K	4	≥320	4	II	TgCkAt62
	7	160	4	II	TgCkAt63
	9	80	4	II	TgCkAt64
	26	20	4	II	TgCkAt65
	39	≥320	4	II	TgCkAt66
	40	160	4	II	TgCkAt67

[a] Of 4 mice inoculated.

3. Results

Antibodies were found in 302 of 830 (36.3%) chickens with titers of 1:10 in 50, 1:20 in 69, 1:40 in 53, 1:80 in 40, 1:160 or higher in 90. In the first batch, antibodies to *T. gondii* were found in only a few chickens (Table 1). In the second batch, *T. gondii* antibodies (MAT titer 1:40 or higher) were found in two of the four farms (Table 1). The seroprevalence was very high on farm H; 125 of 131 (95%) chickens had antibodies and in high titers (Table 1). In the third batch, antibodies to *T. gondii* were found in 113 of 158 sera with titers of 1:10 in 12, 1:20 in 40, 1:40 in 13, 1:80 in 11, 1:160 in 7, and 1:320 or higher in 31.

In total 67 isolates of *T. gondii* were obtained from chickens from Austria; 56 isolates were by bioassay in mice and 11 by bioassay in cats (Tables 2–5). All *T. gondii* isolates were genotype II.

Most mice inoculated with chicken tissues remained asymptomatic, except from chickens from farm H. All mice inoculated with hearts from 38 seropositive chickens, and one-half of the mice inoculated from hearts of another 27 seropositive chickens from farm H died of bacterial infection within 3 days p.i. and they were discarded. From the remainder mice that survived, *T. gondii* was isolated from 27 seropositive chickens by bioassay in mice (Table 4). Only a few tissue cysts were found in the brains of seropositive mice. Tissue cysts were not found in the brain squashes made from about one-fourth of the brain tissue of mice inoculated with hearts from 15 chickens, however, KO mice inoculated with brains of these mice developed pneumonia and tachyzoites were found in lungs of all of the 15 mice (Table 4).

The mice inoculated orally with oocysts of 11 *T. gondii* isolates became ill 5–7 days p.i. and tachyzoites were found in their mesenteric lymph nodes. The mice inoculated with tachyzoites from the mesenteric lymph nodes of these seven isolates remained asymptomatic for 2 months and tissue cysts were found in their brains.

4. Discussion

Little is known of the prevalence of *T. gondii* in commercially-raised chickens. Jacobs and Melton (1966) examined two batches of tissues from 728 fowl processed at a commercial plant in Maryland. In the first batch, ovaries and oviducts from 620 birds were pooled in 12 batches (10 birds per pool) and in the second batch, brain, leg muscle, ovary, and shelled eggs from each of 108 birds were inoculated individually into mice. They found *T. gondii* in 12 pools of ovaries and oviducts in the first batch and from tissues of four chickens from the second batch; ovaries of three and leg muscle of one. Boch et al. (1968) isolated *T. gondii* from the brains of 5 and the heart of 1 of 1636 hens from several farms in Germany. These studies by Jacobs and Melton (1966) and Boch et al. (1968) were performed nearly 40 years ago at a time when the life cycle of *T. gondii* was largely unknown. Jacobs and Melton (1966) had examined ovaries, oviducts and eggs from hens to find out if *T. gondii* can be passed in chicken eggs and serve as a source of infections for humans. Although oviducts and ovaries were found infected with *T. gondii*, there was no evidence for natural *T. gondii* infection in chicken eggs (Jacobs and Melton, 1966). The practice of eating raw eggs by humans is now being discouraged because of the danger of acquiring *Salmonella*.

Recently, Dubey et al. (2004e) found MAT antibodies in 45 of 96 chickens from a commercial farm in Israel and *T. gondii* was isolated from 19 of 45 seropositive chickens. On that farm, cats did not have access to the chicken housing area but were known to defecate in chicken feed stored in open bins (Dubey et al., 2004e). Raising chickens in confinement in wired cages on feed mixed at a central plant reduces chances of *T. gondii* infection. The recent trend of raising chickens free-range in Europe and other countries exposes chickens to *T. gondii* infection. Despite the fact that the birds that we examined were old and probably used for processed food, humans can become infected if proper hygiene is not practiced while dressing the carcasses. Most importantly, the same scenarios might be occurring on other free range organic poultry farms if rodents and cats have access to poultry feed and houses.

The low pathogenicity of *T. gondii* isolates from chickens from Austria to mice was remarkable because none of the mice became ill due to confirmed toxoplasmosis, assuming that none of the mice that died between 1 and 7 days p.i. were infected with *T. gondii*. Additionally, very few tissue cysts were found in the brains of mice inoculated with tissues from these

chickens. Tissue cysts were so few that one-fourth to one-half of the brains of mice had to be examined to find tissue cysts. Historically, *T. gondii* strains have been considered virulent or non virulent based on infections in out-bred mice. Type I strains are considered highly virulent for mice whereas Types II and III strains are considered avirulent (Howe and Sibley, 1995). However, this hypothesis is not applicable to isolates from Brazil; Type III strains from Brazil were mouse virulent whereas those from the USA were avirulent (Dubey et al., 2002, 2003c; Lehmann et al., 2003, 2004). In the present study, seven *T. gondii* isolates were obtained by feeding samples pooled from many chickens. Therefore, it is uncertain if these isolates were single or mixture of *T. gondii* isolates.

The high rate of mortality in mice inoculated with hearts from chickens from farm H was probably related to the isolation method and bacterial infection. Heart digests were inoculated into two mice each instead of the five mice used in other experiments. The level of bacterial contamination might have been higher in the inocula for the two mice inoculated.

In the present study, two cats fed 174 (137 + 37) hearts from seronegative (MAT 1:10 or less) chickens did not shed oocysts, supporting the validity of MAT; cats are highly sensitive to *T. gondii* infection and shed oocysts even after ingesting a few bradyzoites (Dubey, 2001). The threshold MAT titer indicative of *T. gondii* infection in chickens has not been determined. Data comparing serology and recovery of viable *T. gondii* from chickens are now accumulating (Dubey et al., 2002, 2003a,b,c,d,e, 2004b,c, 2005a, in press-b,-c,-d,-e,-f). In the present study, heart was chosen to isolate *T. gondii* because it appears to be the prediction site for the parasite in chickens (Dubey et al., 2004b, in press-e).

The isolates of *T. gondii* from chickens from Austria were genetically different from Brazil. All isolates from Austria were Type II whereas Type II strain has not yet been found in chickens from Brazil. Additionally, most isolates from chickens from Brazil were virulent for mice whereas the isolates from Austria were avirulent.

Acknowledgements

The authors would like to thank S.K. Shen, K. Hopkins, and G. Duscher for technical assistance.

References

Ajzenberg, D., Cogné, N., Paris, L., Bessières, M.H., Thulliez, P., Filisetti, D., Pelloux, H., Marty, P., Dardé, M.L., 2002. Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. J. Infect. Dis. 186, 684–689.

Ajzenberg, D., Bañuls, A.L., Su, C., Dumètre, A., Demar, M., Carme, B., Dardé, M.L., 2004. Genetic diversity, clonality and sexuality in *Toxoplasma gondii*. Int. J. Parasitol. 34, 1185–1196.

Aspinall, T.V., Guy, E.C., Roberts, K.E., Joynson, D.H.M., Hyde, J.E., Sims, P.F.G., 2003. Molecular evidence for multiple *Toxoplasma gondii* infections in individual patients in England and Wales: public health implications. Int. J. Parasitol. 33, 97–103.

Boch, J., Janitschke, K., Rommel, M., 1968. Untersuchungen deutscher Hühnerbestände auf latente *Toxoplasma*-Infektionen. Berl. Münch. Tierärztl. Wochenschr. 81, 90–91.

Boothroyd, J.C., Grigg, M.E., 2002. Population biology of *Toxoplasma gondii* and its relevance to human infection: do different strains cause different disease? Curr. Opin. Microbiol. 5, 438–442.

da Silva, A.V., Pezerico, S.B., de Lima, V.Y., d'Arc Moretti, L., Pinheiro, J.P., Tanaka, E.M., Ribeiro, M.G., Langoni, H., 2005. Genotyping of *Toxoplasma gondii* strains isolated from dogs with neurological signs. Vet. Parasitol. 127, 23–27.

Dubey, J.P., 1995. Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. J. Parasitol. 81, 410–415.

Dubey, J.P., 2001. Oocyst shedding by cats fed isolated bradyzoites and comparision of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. J. Parasitol. 87, 215–219.

Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida, pp. 1–220.

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339.

Dubey, J.P., Lindsay, D.S., 1998. Isolation in immunodeficient mice of *Sarcocystis neurona* from opossum (*Didelphis virginiana*) faeces, and its differentiation from *Sarcocystis falcatula*. Int. J. Parasitol. 28, 1823–1828.

Dubey, J.P., Graham, D.H., Blackston, C.R., Lehmann, T., Gennari, S.M., Ragozo, A.M.A., Nishi, S.M., Shen, S.K., Kwok, O.C.H., Hill, D.E., Thulliez, P., 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo Brazil: Unexpected findings. Int. J. Parasitol. 32, 99–105.

Dubey, J.P., Graham, D.H., Silva, D.S., Lehmann, T., Bahia-Oliveira, L.M.G., 2003a. *Toxoplasma gondii* isolates of free-ranging chickens from Rio de Janeiro, Brazil: mouse mortality, genotype, and oocyst shedding by cats. J. Parasitol. 89, 851–853.

Dubey, J.P., Graham, D.H., Dahl, E., Hilali, M., El-Ghaysh, A., Sreekumar, C., Kwok, O.C.H., Shen, S.K., Lehmann, T., 2003b. Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. Vet. Parasitol. 114, 89–95.

Dubey, J.P., Graham, D.H., Dahl, E., Sreekumar, C., Lehmann, T., Davis, M.F., Morishita, T.Y., 2003c. *Toxoplasma gondii* isolates from free-ranging chickens from the United States. J. Parasitol. 89, 1060–1062.

Dubey, J.P., Navarro, I.T., Graham, D.H., Dahl, E., Freire, R.L., Prudencio, L.B., Sreekumar, C., Vianna, M.C., Lehmann, T., 2003d. Characterization of *Toxoplasma gondii* isolates from free range chickens from Paraná. Brazil. Vet. Parasitol. 117, 229–234.

Dubey, J.P., Venturini, M.C., Venturini, L., Piscopo, M., Graham, D.H., Dahl, E., Sreekumar, C., Vianna, M.C., Lehmann, T., 2003e. Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Argentina. J. Parasitol. 89, 1063–1064.

Dubey, J.P., Graham, D.H., de Young, R.W., Dahl, E., Eberhard, M.L., Nace, E.K., Won, K., Bishop, H., Punkosdy, G., Sreekumar, C., Vianna, M.C.B., Shen, S.K., Kwok, O.C.H., Sumners, J.A., Demarais, S., Humphreys, J.G., Lehmann, T., 2004a. Molecular and biologic characteristics of *Toxoplasma gondii* isolates from wildlife in the United States. J. Parasitol. 90, 67–71.

Dubey, J.P., Levy, M., Sreekumar, C., Kwok, O.C.H., Shen, S.K., Dahl, E., Thulliez, P., Lehmann, T., 2004b. Tissue distribution and molecular characterization of chicken isolates of *Toxoplasma gondii* from Peru. J. Parasitol. 90, 1015–1018.

Dubey, J.P., Morales, E.S., Lehmann, T., 2004c. Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Mexico. J. Parasitol. 90, 411–413.

Dubey, J.P., Parnell, P.G., Sreekumar, C., Vianna, M.C.B., de Young, R.W., Dahl, E., Lehmann, T., 2004d. Biologic and molecular charactaeristics of *Toxoplasma gondii* isolates from striped skunk (*Mephitis mephitis*), Canada goose (*Branta canadensis*), blacked-winged lory (*Eos cyanogenia*), and cats (*Felis catus*). J. Parasitol. 90, 1171–1174.

Dubey, J.P., Salant, H., Sreekumar, C., Dahl, E., Vianna, M.C.B., Shen, S.K., Kwok, O.C.H., Spira, D., Hamburger, J., Lehmann, T., 2004e. High prevalence of *Toxoplasma gondii* in a commercial flock of chickens in Israel, and public health implications of free-range farming. Vet. Parasitol. 121, 317–322.

Dubey, J.P., Karhemere, S., Dahl, E., Sreekumar, C., Diabaté, A., Dabiré, K.R., Vianna, M.C.B., Kwok, O.C.H., Lehmann, T., 2005a. First biologic and genetic characterization of *Toxoplasma gondii* isolates from chickens from Africa (Democratic Republic of Congo, Mali, Burkina Faso, and Kenya). J. Parasitol. 91, 69–72.

Dubey, J.P., Lopez, B., Alveraz, M., Mendoza, C., Lehmann, T., in press-b. Isolation, tissue distribution, and molecular characterization of *Toxoplasma gondii* from free-range chickens from Guatemala. J. Parasitol.

Dubey, J.P., Marcet, P.L., Lehmann, T., in press-c. Characterization of *Toxoplasma gondii* isolates from free-range chickens in Argentina. J. Parasitol.

Dubey, J.P., Rajapakse, R.P.V.J., Ekanayake, D.K., Sreekumar, C., Lehmann, T., in press-d. Isolation and molecular characterization of *Toxoplasma gondii* from chickens from Sri Lanka. J. Parasitol.

Dubey, J.P., Bhaiyat, M.I., de Allie, C., Macpherson, C.N.L., Sharma, R.N., Sreekumar, C., Vianna, M.C.B., Shen, S.K., Kwok, O.C.H., Lehmann, T., in press-e. Isolation, tissue distribution, and molecular characterization of *Toxoplasma gondii* from chickens from Grenada, West Indies. J. Parasitol.

Dubey, J.P., Lenhart, A., Castillo, C.E., Alvarez, L., Marcet, P., Sreekumar, C., Lehmann, T., in press-f. *Toxoplasma gondii* infections in chickens from Venezuela: isolation, tissue distribution, and molecular characterization. J. Parasitol.

Fuentes, I., Rubio, J.M., Ramírez, C., Alvar, J., 2001. Genotypic characterization of *Toxoplasma gondii* strains associated with human toxoplasmosis in Spain: direct analysis from clinical samples. J. Clin. Microbiol. 39, 1566–1570.

Grigg, M.E., Ganatra, J., Boothrooyd, J.C., Margolis, T.P., 2001. Unusual abundance of atypical strains associated with human ocular toxoplasmosis. J. Infect. Dis. 184, 633–639.

Howe, D.K., Sibley, L.D., 1995. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. J. Infect. Dis. 172, 1561–1566.

Howe, D.K., Honoré, S., Derouin, F., Sibley, L.D., 1997. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. J. Clin. Microbiol. 35, 1411–1414.

Jacobs, L., Melton, M.L., 1966. Toxoplasmosis in chickens. J. Parasitol. 52, 1158–1162.

Jungersen, G., Jensen, L., Rask, M.R., Lind, P., 2002. Non-lethal infection parameters in mice separate sheep type II *Toxoplasma gondii* isolates by virulence. Comp. Immunol. Microbiol. Infect. Dis. 25, 187–195.

Lehmann, T., Blackston, C.R., Parmley, S.F., Remington, J.S., Dubey, J.P., 2000. Strain typing of *Toxoplasma gondii*: comparison of antigen-coding and housekeeping genes. J. Parasitol. 86, 960–971.

Lehmann, T., Graham, D.H., Dahl, E., Sreekumar, C., Launer, F., Corn, J.L., Gamble, H.R., Dubey, J.P., 2003. Transmission dynamics of *Toxoplasma gondii* on a pig farm.. Infect. Genet. Evol. 3, 135–141.

Lehmann, T., Graham, D.H., Dahl, E.R., Bahia-Oliveira, L.M.G., Gennari, S.M., Dubey, J.P., 2004. Variation in the structure of *Toxoplasma gondii* and the roles of selfing, drift, and epistatic selection in maintaining linkage disequilibria. Infect. Genet. Evol. 4, 107–114.

Mondragon, R., Howe, D.K., Dubey, J.P., Sibley, L.D., 1998. Genotypic analysis of *Toxoplasma gondii* isolates from pigs. J. Parasitol. 84, 639–641.

Owen, M.R., Trees, A.J., 1999. Genotyping of *Toxoplasma gondii* associated with abortion in sheep. J. Parasitol. 85, 382–384.

Sreekumar, C., Graham, D.H., Dahl, E., Lehmann, T., Raman, M., Bhalerao, D.P., Vianna, M.C.B., Dubey, J.P., 2003. Genotyping of *Toxoplasma gondii* isolates from chickens from India. Vet. Parasitol. 118, 187–194.